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BRIEF COMMUNICATION

Effects of silicon sources on its deposition, chlorophyll content, and disease and pest resistance in rice

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Abstract

Rice (*Oryza sativa* L.) was grown in pots with pyridine N-oxide (PNO), 4-morpholino pyridine N-oxide (MNO), and sodium meta silicate as the sources for silicon. Aliquots of these were added in fortnightly intervals to seedlings through anthesis stage. The plants were monitored for plant growth characteristics, chlorophyll content (SPAD values), photosystem 2 activity (variable to maximum fluorescence ratio of dark adapted leaves), and for blast and yellow stem borer resistance. Deposition of silica in the leaves was monitored by scanning electron microscopy and silicon mapping. PNO or MNO application resulted in significant silicon accumulation in leaf bundle sheath cells. Application of PNO and MNO imparted disease and pest resistance by increasing silicon uptake of rice plants.

Additional key words: chlorophyll fluorescence induction, 4-morpholino pyridine N-oxide (MNO), *Oryza sativa*, photosystem 2 efficiency, pyridine N-oxide, silicon accumulation.

Silicon is beneficial particularly in graminaeceous plants, but is not an essential element (Marschner 1988). Application of Si sustains rice yields in Japan (Takahashi and Mayak 1990) and in Florida (Datnoff 1994). The ability of silicon in avoiding disease and pest (Ma and Takahashi 1990, Winslow 1992, Epstein 1994, Wang *et al.* 1994, Hodson and Evans 1995, Datnoff *et al.* 1997, Deren 1997, Kim *et al.* 2002, Rodrigues *et al.* 2003; for review see Ma 2004) point towards the significant role played by Si in various plant processes. To the list of silicon solubilizing compounds such as polymer oxides (Wynn Parry 1978), fused calcium silicate (Alvarez *et al.* 1985), pyridine N-oxide (PNO) and 4-morpholino pyridine N-oxide (MNO) (Chandrasekher Rao 2002, Ranganathan *et al.* 2004) have recently been added.

The silicon uptake using metal salts of silicic acid requires their hydrolysis prior to their uptake. In either case they would affect the ionic balance of the system. The proposed mechanism for the solubilization of silica by PNO or MNO is novel and probably involves polarization of surface silica layer through interaction with the oxygen of the pyridine N-oxides. In the solubilization, PNO and MNO are regenerated, as evidenced by the fact that clear water containing freshly prepared PNO/MNO-silica complexes slowly deposits granular silica (Chandrasekher Rao 2002, Ranganathan *et al.* 2004). We tested the effect of PNO and MNO on rice with focus on their ability to enhance the plant silicon content, and disease and pest resistance.

Rice (*Oryza sativa* L. cv. Rasi) plants were grown in pots (45 cm diameter), 3 plants per pot with the recommended (80:40:20 NPK) fertilizer dose. Nitrogen fertilizer was applied as three splits as recommended for rice cultivation. In six separate batches, consisting of ten pots per batch, PNO (150 mg kg^{-1}), PNO (100 mg kg^{-1}), MNO (150 mg kg^{-1}), MNO (100 mg kg^{-1}), and sodium metasilicate, SMS (100 and 150 mg kg^{-1}) were added at fortnightly intervals throughout the growth period. The pH and electrical conductivity of the soil ranged 7.5 - 6.5 and 0.6 - 0.9 mS, respectively.

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Abbreviations: Chl - chlorophyll; F_m - maximum fluorescence of chlorophyll *a*; F_v - variable fluorescence; MNO - 4-morpholino pyridine N-oxide; PNO - pyridine N-oxide; PS 2 - photosystem 2.
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Leaf length, width, total dry matter were measured at anthesis stage. Simultaneously, chlorophyll content was measured in leaves by *SPAD* meter (*Minolta*, Tokyo, Japan) and efficiency of photosystem 2 (PS 2) was estimated according to variable to maximum fluorescence ratio (F_v/F_m) measured on dark adapted leaves using a fluorescence meter (*ADC Fm 1500*, Herts, UK) .

The blast pathogen (*Pyricularia grisea*) was isolated and the plants grown with the above silicon treatments were inoculated twice. The plants were examined for rice blast disease 15 d after inoculation. Chl content and F_v/F_m were also measured in the flag leaf under all the treatments.

Yellow stem borer resistance was studied by releasing 30 neonate yellow stem borer larvae, *Scripophaga incertulus* onto each plant in a pot. Observations were recorded 10 d after release of the larvae when the symptoms of damage manifested as either dead hearts or white ears. Percent total damage was derived as [(number of dead hearts + number of white ears) / total number of tillers] × 100.

For scanning electron micrograph (SEM) pictures, the leaves were cut to 2 cm in length, wiped with tissue paper to remove moisture, taped to aluminum stubs, sputter coated with carbon and then loaded on to the instrument. Energy dispersive X-ray micro analysis (EDX) was carried out with an accelerating voltage of 15 kV. The surface scan was performed on an integrated analytical SEM (*Philips*, The Netherlands) with energy EDX attachment. The SEM was fitted with advanced image analysis software for detecting silicon maps.

Plants receiving PNO or MNO, or silicon in the form of SMS were phenotypically stronger than control plants. There was a significant increase in leaf length and width, particularly with MNO and SMS (Table 1).

The addition of PNO or MNO and SMS had little influence on the SPAD values and F_v/F_m in control plants (Table 1). However, significant differences in Chl amount and PS 2 activity were observed in blast infected leaves in response to silicon solubilizers as well as SMS. Blast infestation of leaves brought about by inoculation with *P. grisea* led to a marked decrease in SPAD values and PS 2 activity of the untreated plants. On the other hand, plants treated with PNO, MNO or SMS showed only marginal decrease in these characteristics (Table 1). Although silicon benefits the rice crop by increasing resistance to fungi (Rodrigues *et al.* 2003), the probable mechanism underlying this phenomenon is less understood. The increased resistance to blast is probably associated with cell wall fortification (Kim *et al.* 2002). The Si mediated rice blast resistance correlates with specific cell reaction that interfers with the development of the fungus (Rodrigues *et al.* 2003). We found that the addition of PNO and MNO or SMS restored Chl content and PS 2 activity in the infected rice leaves. The treated plants had lower disease incidence compared to controls.

Earlier experiments in our laboratory with PNO and MNO showed enhanced solubility of silicon in water at pH 6 - 8, possibly by polarizing the interface Si through interaction with oxygen of PNO and MNO *via* penta oxygen coordinated intermediates (Chandrasekher Rao 2002). Such enhancement of solubility of silica in water was followed by colorimetric silicon estimation: in the absence of any reagent the background value was 204 μM, on addition of 0.1 g of PNO or MNO the values were 292 and 390 μM, respectively, The solubilization of soil silicon in the form of SiO_2 proceeds slowly by hydration within a narrow pH range and saturation solubility is only 10^{-4} M.

The addition of silicon lead also to a reduction of damage due to yellow stem borer (Table 1). This could be due to a lower preference as well as digestibility of the leaves and straw by the insect owing to the presence of higher silicon content. These results are in conformity with similar studies on disease and pest resistance on rice and in other crops (Hodson and Evans 1995, Datnoff *et al.* 1997).

The suggestion that enhanced silica deposition is linked to the ability of the rice plant to combat biotic stresses is further supported by SEM and EDX analysis of silicon distribution in the rice leaves. In the absence of added silicon, the leaves exhibited a scattered profile of silicon distribution (Fig. 1). The leaves treated with MNO, PNO or SMS showed enhanced silicon content and localization of silicon bodies in leaf bundle sheath cells, particularly in the primary and secondary cell wall.

Table 1. Influence of silicon solubilizers on leaf and stem morphological parameters and SPAD and F_v/F_m values in rice, healthy and infected by *Pyricularia*, and on damage caused by yellow stem borer.

Treatment [mg kg^{-1}]	Leaf length [cm]	Leaf width [cm]	Leaf dry mass [g]	Stem dry mass [g]	Leaf area [m^2 $plant^{-1}$]	SPAD values healthy	SPAD values infected	F_v/F_m healthy	F_v/F_m infected	YSB damage [%]
Control	40.4	1.80	2.70	3.13	5.61	41.15	27.65	0.778	0.636	84.8±1.5
PNO 150	55.9	1.80	3.43	3.21	6.92	38.05	36.90	0.730	0.732	-
MNO 150	48.4	1.60	3.93	2.95	8.16	43.35	42.35	0.789	0.760	58.9±7.9
MNO 100	54.5	2.00	5.57	4.95	11.44	39.35	41.95	0.762	0.736	46.1±9.5
SiO_2 150	50.2	1.70	5.26	4.98	11.95	39.90	39.95	0.761	0.761	59.3±8.7
SiO_2 100	54.2	1.85	5.23	5.98	11.11	43.10	41.55	0.670	0.772	49.6±9.7
$LSD_{0.05}$	2.2	0.07	NS	2.06	NS	2.30	2.80	0.016	0.017	

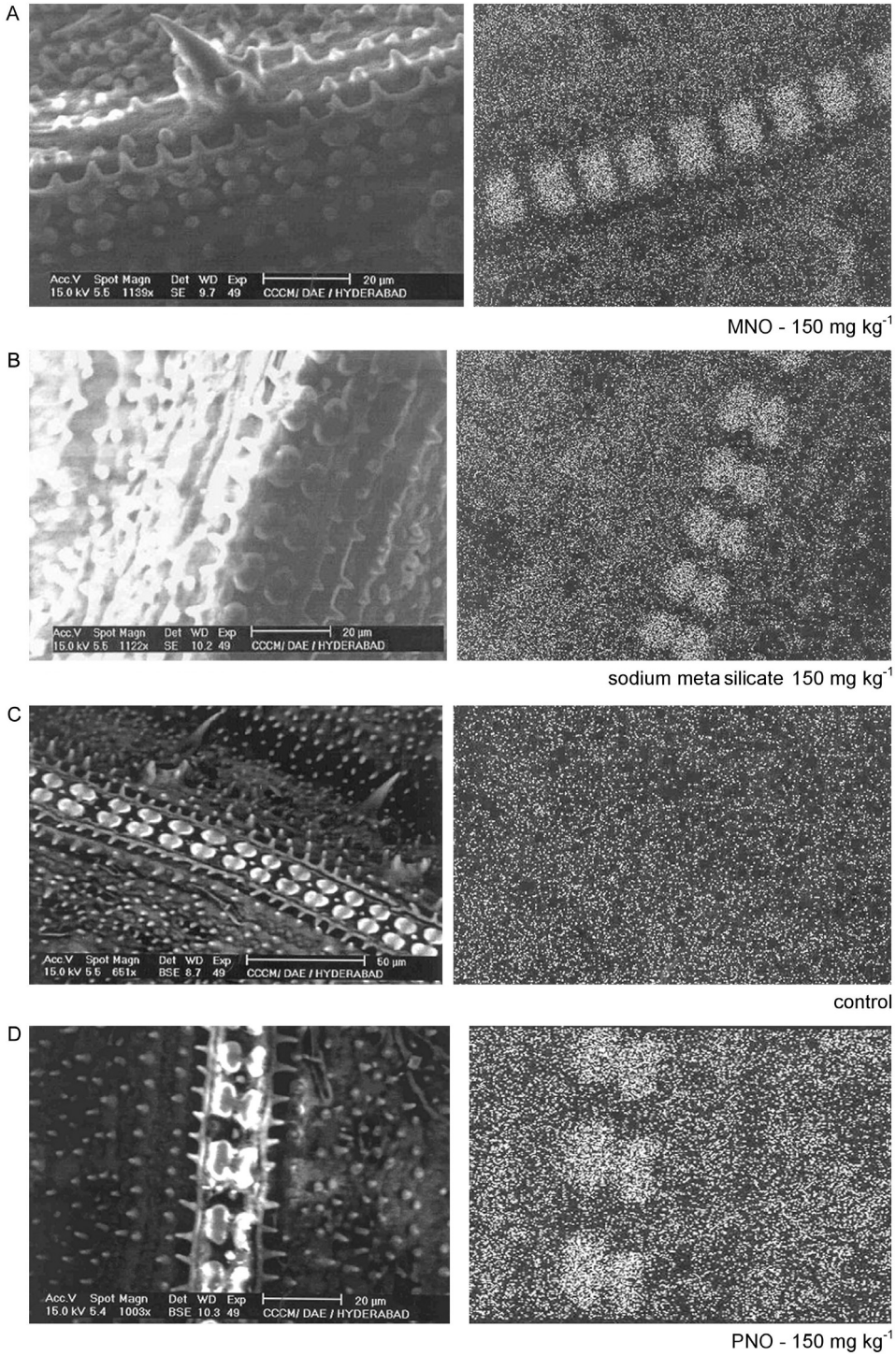

Fig. 1. Scanning electron micrograph of silicon mapping (*right*) and its corresponding bundle sheath cells (*left*). Application of *A* - 150 mg kg^{-1} MNO, *B* - 150 mg kg^{-1} sodium silicate, *C* - control, and *D* - 150 mg kg^{-1} PNO.

Electron microscopy and *in situ* X-ray analysis of rice leaves reflect the differences in silicon distribution and cell wall structure between silicon treated and untreated plants (Balasta *et al.* 1989, Kim *et al.* 2002, Rodrigues *et al*. 2003). The SEM pictures show that PNO and MNO enhanced the silica deposition on the leaves of rice plants concomitant with the localization of silicon bodies in leaf bundle sheath cells and in the primary and secondary cell walls.

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